Effects of Rumen-Protected Methionine on Rumen Microbial Protein Production, Milk Production Performance, and Nitrogen Excretion in Dairy Cows: Postprint

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Abstract

This experiment aimed to investigate the effects of rumen-protected methionine (RPMet) on rumen microbial protein (MCP) yield, milk performance, and nitrogen excretion in dairy cows. Forty Holstein dairy cows with similar age, body weight, parity, milk yield, milk composition, and lactation period [(90$±$15) d] were selected and randomly divided into 4 groups with 10 cows per group. The control group and experimental groups 1, 2, and 3 were supplemented with 0, 15, 25, and 35 g/(d · head) RPMet, respectively. The preliminary period was 15 d, and the formal experimental period was 60 d. The results showed: 1) The MCP yield in the rumen of all experimental groups was extremely significantly higher than that of the control group (P<0.01), with experimental groups 1, 2, and 3 showing increases of 13.10%, 20.45%, and 16.23% compared to the control group, respectively. 2) The milk yield of the experimental groups was significantly or extremely significantly higher than that of the control group (P<0.05 or P<0.01), with experimental groups 1, 2, and 3 showing increases of 8.12%, 13.32%, and 10.32% compared to the control group, respectively; RPMet could significantly or extremely significantly increase milk fat percentage and milk protein percentage (P<0.05 or P<0.01), and significantly or extremely significantly reduce milk somatic cell count (P<0.05 or P<0.01), with experimental group 2 being the lowest. 3) In terms of total nitrogen excretion, all experimental groups were extremely significantly lower than the control group (P<0.01), with experimental groups 1, 2, and 3 showing reductions of 8.55%, 17.49%, and 13.25% compared to the control group, respectively. It can be concluded that under the conditions of this experiment, dietary supplementation of RPMet could significantly increase MCP yield in the rumen of dairy cows, reduce nitrogen excretion, and improve production performance in dairy cows. Based on comprehensive evaluation of all experimental indicators, the optimal

supplementation level of RPMet was 25 g/(d · head).

Full Text

Effects of Rumen-Protected Methionine on Ruminal Microbial Protein Production, Milk Performance and Nitrogen Excretion in Dairy Cows

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Abstract

This experiment was conducted to investigate the effects of rumen-protected methionine (RPMet) on ruminal microbial protein (MCP) production, milk performance, and nitrogen excretion in dairy cows. Forty Holstein cows with similar age, body weight, parity, milk yield, milk composition, and lactation stage [(90$±$15) d] were randomly allocated into four groups (n=10). The control group and test groups 1, 2, and 3 received RPMet supplementation at 0, 15, 25, and 35 g/(d · head), respectively. The experiment consisted of a 15-day preliminary period followed by a 60-day formal trial period. The results showed: (1) MCP production in all test groups was extremely significantly higher than in the control group (P<0.01), with increases of 13.10%, 20.45%, and 16.23% for test groups 1, 2, and 3, respectively. (2) Milk yield in test groups was significantly or extremely significantly higher than in the control group (P<0.05 or P<0.01), with increases of 8.12%, 13.32%, and 10.32% for groups 1, 2, and 3, respectively. RPMet supplementation significantly or extremely significantly increased milk fat and protein percentages (P<0.05 or P<0.01) while significantly reducing milk somatic cell counts (P<0.05 or P<0.01), with test group 2 showing the lowest values. (3) Total nitrogen excretion in all test groups was extremely significantly lower than in the control group (P<0.01), with reductions of 8.55%, 17.49%, and 13.25% for groups 1, 2, and 3, respectively. These findings indicate that dietary RPMet supplementation can significantly enhance ruminal MCP production, reduce nitrogen excretion, and improve dairy cow performance under the experimental conditions. The optimal RPMet supplementation level was 25 g/(d · head).

Keywords: rumen-protected methionine; ruminal microbial protein; milk performance; nitrogen excretion

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Introduction

In recent years, with the increasing intensification and scaling of dairy farming in China, rising feed costs and environmental pollution have become critical factors constraining the sustainable development of the dairy industry. During ruminant production, the shortage of protein feed ingredients and their low utilization efficiency represent major limiting factors for animal performance. Improving protein feed utilization, reducing feeding costs, and decreasing nitrogen excretion are therefore of great significance for dairy industry development. Amino acid nutrition constitutes the essence and core of protein nutrition in ruminants, and nitrogen utilization efficiency can be enhanced while nitrogen excretion reduced through amino acid supplementation. Methionine serves as the first or second limiting amino acid for ruminants and is particularly crucial for high-yielding dairy cows. To prevent methionine degradation in the rumen, protective treatments using physical or chemical modification methods can be applied to enable rumen-protected absorption.

Previous research has demonstrated various benefits of rumen-protected methionine (RPMet) supplementation. Zou et al. found that RPMet supplementation in dairy cow diets significantly increased milk yield, milk protein percentage, and milk specific gravity during early lactation, while also improving milk fat percentage and non-fat solids content. Yan reported that RPMet supplementation reduced urinary urea nitrogen excretion, the ratio of urea nitrogen to nitrogen intake, and total urinary nitrogen loss, while increasing nitrogen deposition and biological value in Small-tailed Han sheep. In vitro studies by Salsbury et al. indicated that methionine and methionine hydroxy analog (MHA) could accelerate microbial growth. Han et al. observed that supplementing 30 g/(d · head) of palm oil-coated methionine met the methionine requirements of rumen microorganisms, improved NH_3-N utilization, and promoted MCP synthesis. However, current research on RPMet in dairy cows has primarily focused on milk yield, with limited and inconsistent reports on its effects on ruminal MCP production and nitrogen excretion. This experiment investigated the effects of different RPMet supplementation levels on ruminal MCP production, milk performance, and nitrogen excretion in dairy cows, aiming to improve protein feed utilization and milk performance while reducing feeding costs and nitrogen excretion, thereby providing a reference for sustainable dairy farming development in China.

Materials and Methods

1.1 RPMet Product

The RPMet product was purchased from Qingdao Runbot Biotechnology Co., Ltd., appearing as white granular material composed of DL-methionine, palm oil, and silicon dioxide, with DL-methionine content $ $60% and moisture $ $12%.

1.2 Experimental Design

This experiment employed a single-factor randomized block design. Forty Holstein cows from Qingdao Aote Dairy Farm with similar age, body weight, parity, milk yield, milk composition, and lactation stage [(90$±$15) d] were randomly divided into four groups (n=10). The control group and test groups 1, 2, and 3 received RPMet supplementation at 0, 15, 25, and 35 g/(d · head), respectively. Each cow was provided with 0.5 kg of concentrate daily as a carrier mixed with RPMet. The remaining concentrate was blended with roughage to formulate a total mixed ration (TMR). The RPMet was thoroughly mixed with the concentrate carrier and fed with the TMR. The TMR composition and nutrient levels are presented in Table 1 .

The total experimental period was 75 days, including a 15-day preliminary period and a 60-day formal trial period. Cows were housed in a barn, milked twice daily (04:00 and 16:00) using a DeLaval milking system, and fed TMR twice daily (04:30 and 16:30). Cows had access to TMR for over 20 hours per day. After feeding, cows had free access to water and exercise in a paddock. Routine deworming, lighting, and management practices were applied according to standard procedures.

1.3 Sample Collection

1.3.1 Feed Samples TMR samples were collected using the quartering method, dried at 65 °C to produce air-dried samples, and ground for subsequent analysis.

1.3.2 Fecal Samples Fecal samples were collected three times during the preliminary period (days 1-3), formal trial period (days 28-30), and late trial period (days 58-60) using the total fecal collection method. For three consecutive days, complete 24-hour fecal collections were performed for all 10 cows in each group. Before collection, cow beds were thoroughly cleaned. Fresh feces were collected promptly, mixed daily, and weighed. Using the quartering method, daily fecal samples were collected and treated with 25 mL of 10% sulfuric acid per 100 g of feces for nitrogen fixation, then stored at -20 °C. On the final sampling day, the three-day samples were proportionally mixed by weight, dried at 65 °C to constant weight, and stored for nitrogen content analysis.

1.3.3 Urine Samples Urine samples were collected three times during the same periods as fecal collection, following the spot urine collection method described by Zhu. Samples were obtained through manual collection combined with bladder catheterization. Cows were restrained using neck clamps, and catheters were inserted into the bladder to collect urine sequentially. If a cow urinated spontaneously during collection, personnel assisted in collection. Urine was collected twice daily at 12-hour intervals for three consecutive days, with collection times delayed by 4 hours each day. Collected urine was treated with 98% concentrated sulfuric acid to adjust pH (pH<3) and stored at -20 °C.

1.3.4 Milk Samples Milk samples were collected on day 1 of the preliminary period and every 15 days during the formal trial period. Samples were collected proportionally to morning and evening milk yields, totaling 65 mL per collection. For milk composition analysis, 50 mL of milk was preserved with potassium dichromate (0.6 mg/mL), mixed thoroughly, and refrigerated at 4 °C. The remaining 15 mL was centrifuged at 1,500×g for 10 minutes. Four milliliters of the supernatant was mixed with an equal volume (4 mL) of 25% trichloroacetic acid (TCA), allowed to stand for 5 minutes, then centrifuged at 3,500×g for 20 minutes to remove milk proteins. The processed sample (1.5 mL) was stored at -20 °C for milk urea nitrogen determination.

1.4 Measurements and Calculations

1.4.1 Feed Intake During the preliminary period, TMR refusals were weighed every 2 days, with feed delivery amounts recorded (displayed on the TMR mixer). Refusals from the previous feeding were collected and weighed before each new feeding. Six records were obtained to calculate average feed intake per cow during the preliminary period. The same method was applied during the formal trial period, with intake recorded every 10 days (six total records) to calculate average intake. Feed delivery amounts were adjusted based on these records. Main nutrient intakes were calculated based on average intake and TMR nutrient composition.

1.4.2 MCP Production Urinary purine derivatives (PD) originate primarily from rumen microbial purines, enabling MCP production estimation through PD excretion measurement. Colorimetric methods were used to determine urinary uric acid and allantoin concentrations, with their sum representing total PD excretion.

The amount of exogenous purines absorbed by the small intestine (X) was calculated using the formula:

$$Y = 0.85X + 0.385BW^{0.75}$$

where Y is urinary PD excretion (mmol/d), 0.85 is the recovery rate of absorbed purines converted to urinary PD in cattle, 0.385 is endogenous PD excretion when purine absorption is zero, and BW^{0.75} is metabolic body weight.

MCP production was calculated as:

$$MCP(g/d) = \frac{6.25 \times 70 \times X}{0.83 \times 0.116 \times 1000} = 6.25 \times 0.727X$$

where X is the amount of exogenous purines absorbed by the small intestine (mmol/d), 70 is the nitrogen content per mole of purine (mg/mol), 0.83 is the digestibility of microbial nucleic acid purines, 0.116 is the proportion of purine nitrogen in total rumen microbial nitrogen, and 6.25 is the conversion coefficient from nitrogen to protein. Formal trial period MCP production was calculated as the average of values obtained on days 30 and 60.

1.4.3 Milk Yield and Composition Milk yield was recorded automatically by the DeLaval herringbone milking system. During both preliminary and formal periods, milk yield was recorded every 5 days (three consecutive days each time) and averaged. Milk fat percentage, protein percentage, lactose percentage, and somatic cell counts were determined using a CombiFoss FT+ automatic analyzer (Foss, Denmark) at the Dairy Performance Testing Laboratory of Shandong Academy of Agricultural Sciences. Weighted averages were used to calculate milk composition during the formal trial period.

1.4.4 Nitrogen Metabolism Indices Urinary nitrogen content was analyzed using the Kjeldahl method, urinary urea nitrogen by the urease method, and urinary creatinine by the picric acid colorimetric method, with kits purchased from Nanjing Jiancheng Bioengineering Institute. Following Valadares et al., urinary creatinine (approximately 29 mg excreted per kg body weight daily) was used as a marker to estimate urine volume. Crude protein (CP) content in diets and feces was determined according to methods described by Zhang. Nitrogen metabolism indices were calculated as follows:

- Fecal nitrogen (g/d) = daily nitrogen excretion × fecal CP content × 0.16
- Milk nitrogen (g/d) = milk yield × milk protein percentage × 0.16
- Digestible nitrogen (g/d) = nitrogen intake -fecal nitrogen
- Total nitrogen excretion (g/d) = fecal nitrogen + urinary nitrogen
- Nitrogen apparent digestibility (%) = [(nitrogen intake -fecal nitrogen) / nitrogen intake] × 100

1.5 Data Processing and Analysis

Data were initially processed using Excel 2016. SPSS 20.0 software was used for one-way ANOVA, with Duncan' s multiple comparison test used to examine differences between groups. Significance was declared at $P<0.05$ and extreme significance at $P<0.01$. Results are expressed as means ± standard error.

Results

2.1 Effects of RPMet Supplementation Level on Main Nutrient Intake in Dairy Cows

As shown in Table 2 , RPMet supplementation had minimal effects on dry matter and other nutrient intakes.

2.2 Effects of RPMet Supplementation Level on Ruminal MCP Production in Dairy Cows

Table 3 shows that urinary uric acid and allantoin excretion in all test groups were extremely significantly higher than in the control group (P<0.01), with test group 2 being significantly higher than test group 1 (P<0.05) but not significantly different from test group 3 (P>0.05). Total purine derivative excretion in all test groups was extremely significantly higher than in the control group (P<0.01), with test group 2 significantly higher than test group 1 (P<0.05) but not significantly different from test group 3 (P>0.05). MCP production in all test groups was extremely significantly higher than in the control group (P<0.01), with test group 2 significantly higher than test group 1 (P<0.05) but not significantly different from test group 3 (P>0.05). Compared with the control group, MCP production increased by 13.10%, 20.45%, and 16.23% in test groups 1, 2, and 3, respectively.

2.3 Effects of RPMet Supplementation Level on Milk Yield and Composition in Dairy Cows

Table 4 indicates no significant differences in milk yield or composition among groups during the preliminary period (P>0.05). During the formal trial period, milk yield in test groups 2 and 3 was extremely significantly higher than in the control group (P<0.01), while test group 1 was significantly higher (P<0.05). No significant differences were observed among test groups (P>0.05). Compared with the control group, milk yield increased by 8.12%, 13.32%, and 10.32% in test groups 1, 2, and 3, respectively.

For milk fat percentage, test groups 2 and 3 were extremely significantly higher than the control group (P<0.01), with test group 1 significantly higher (P<0.05). Test group 2 was extremely significantly higher than test group 1 (P<0.01), while no significant differences were found between test group 3 and the other test groups (P>0.05). Milk protein percentage in all test groups was extremely significantly higher than in the control group (P<0.01), with test group 2 extremely significantly higher than test group 1 (P<0.01) but not significantly different from test group 3 (P>0.05). No significant differences in lactose percentage were observed among any groups (P>0.05). Milk somatic cell counts in test groups 2 and 3 were extremely significantly lower than in the control group (P<0.01), with test group 2 significantly lower than test group 1 (P<0.05) but not significantly different from test group 3 (P>0.05). Test group 1 was significantly lower than the control group (P<0.05) but not significantly different from test group 3 (P>0.05).

2.4 Effects of RPMet Supplementation Level on Nitrogen Apparent Digestibility and Excretion in Dairy Cows

Table 5 shows no significant differences in nitrogen intake among groups (P>0.05). Fecal nitrogen excretion in test groups 2 and 3 was extremely significantly lower than in the control group (P<0.01) and significantly lower than in test group 1 (P<0.05), with no significant difference between test groups 2 and 3 (P>0.05). Test group 1 was significantly lower than the control group (P<0.05).

Urinary nitrogen excretion in all test groups was extremely significantly lower than in the control group (P<0.01), with test group 2 extremely significantly lower than test group 1 (P<0.01) but not significantly different from test group 3 (P>0.05). Milk nitrogen excretion in all test groups was extremely significantly higher than in the control group (P<0.01), with test group 2 extremely significantly higher than test group 1 (P<0.01) but not significantly different from test group 3 (P>0.05).

For milk urea nitrogen excretion, test group 2 was extremely significantly lower than the control group (P<0.01) and significantly lower than test group 1 (P<0.05), but not significantly different from test group 3 (P>0.05). Test group 3 was significantly lower than the control group (P<0.05) but not significantly different from test group 1 (P>0.05), while test group 1 did not differ significantly from the control group (P>0.05).

Total nitrogen excretion was reduced by 8.55%, 17.49%, and 13.25% in test groups 1, 2, and 3, respectively, compared with the control group. All test groups were extremely significantly lower than the control group (P<0.01), with test group 2 extremely significantly lower than test group 1 (P<0.01) but not significantly different from test group 3 (P>0.05). Test group 3 was significantly lower than test group 1 (P<0.05).

Digestible nitrogen in test groups 2 and 3 was extremely significantly higher than in the control group (P<0.01), with test group 2 significantly higher than test group 1 (P<0.05) but not significantly different from test group 3 (P>0.05). Test group 1 was significantly higher than the control group (P<0.05) but not significantly different from test group 3 (P>0.05). Nitrogen apparent digestibility in test groups 2 and 3 was extremely significantly higher than in the control group (P<0.01) and significantly higher than in test group 1 (P<0.05), with no significant difference between test groups 2 and 3 (P>0.05). Test group 1 was significantly higher than the control group (P<0.05).

Discussion

3.1 Effects of RPMet Supplementation Level on Nutrient Intake in Dairy Cows

Previous studies have reported consistent findings regarding RPMet' s minimal impact on feed intake. Wu et al. found that ruminal infusion of RPMet (N-acetyl-DL-methionine) did not significantly affect dry matter intake in sheep. Zou et al. similarly observed no significant effect of dietary RPMet on dry matter intake in lactating buffaloes. Yang reported that ruminal infusion of animal fat-coated methionine significantly improved nitrogen deposition and apparent digestibility in beef cattle. Dietary RPMet supplementation increases methionine content in the duodenum, effectively reducing urinary urea nitrogen content and nitrogen loss, thereby improving nitrogen deposition rate and utilization efficiency. These findings align with our results showing that RPMet supplementation did not significantly affect feed intake but significantly improved nitrogen apparent digestibility and reduced nitrogen excretion in dairy cows.

3.2 Effects of RPMet Supplementation Level on Ruminal MCP Production in Dairy Cows

Microbial crude protein provides 60–70% of the protein requirements for ruminants and represents the most important nitrogen source. MCP production reflects not only microbial nitrogen utilization efficiency but also indirectly indicates rumen microbial population size. Berthiaume et al. reported that feeding stearic acid-coated methionine to non-lactating Holstein cows reduced duodenal ammonia nitrogen by 6% while increasing MCP flow by 38% and duodenal methionine content by 50%. Gao found that RPMet supplementation in sheep diets enhanced rumen microbial activity and NH_3-N utilization, thereby increasing MCP production. Our results demonstrate that dietary RPMet supplementation significantly increased ruminal MCP production, consistent with these previous findings.

RPMet possesses dual characteristics of rumen protection and post-ruminal release, enabling most of the product to safely bypass rumen degradation while being rapidly and effectively released in the lower digestive tract for biological absorption. The small amount of free methionine released in the rumen improves the rumen environment and promotes microbial growth and reproduction. Xiong reported that dietary supplementation or ruminal infusion of N-hydroxymethyl methionine calcium (N-HMM-Ca) could meet the methionine requirements of rumen microorganisms, improve NH_3-N utilization, and increase MCP synthesis. Han et al. also observed that dietary RPMet supplementation significantly reduced NH_3-N concentration. The significant reduction in ruminal NH_3-N concentration indicates that RPMet improves microbial NH_3-N utilization, leading to increased MCP synthesis.

3.3 Effects of RPMet Supplementation Level on Milk Yield and Composition in Dairy Cows

Han et al. reported that supplementing 12 g/d RPMet during summer improved milk yield, milk fat percentage, milk protein percentage, and lactose percentage while reducing somatic cell counts. Zou et al. found that RPMet supplementation significantly increased milk yield and milk protein percentage during early lactation, with improvements in milk fat percentage. During peak lactation, milk protein percentage is typically low because the increase in dry matter intake lags behind the rise in milk yield, resulting in negative energy balance. Direct methionine supplementation is largely degraded by rumen microorganisms, with minimal reaching the small intestine for absorption, negating its intended effect. Therefore, RPMet supplementation during early lactation increases absorbable methionine in the small intestine and improves methionine utilization efficiency.

Methionine serves not only as a protein synthesis substrate but also provides methyl groups to promote lipoprotein synthesis, which facilitates the transport of hepatically synthesized plasma triglycerides to the mammary gland. Additionally, the rumen-protected fat component in RPMet provides substrates for milk fat synthesis. These combined effects enhance milk fat percentage. Our results demonstrate that dietary RPMet supplementation significantly increased milk yield, milk fat percentage, and milk protein percentage while significantly reducing milk somatic cell counts. Somatic cell count is an important indicator of mammary gland health; higher counts indicate greater risk of mastitis, while reduced counts reflect improved udder health.

3.4 Effects of RPMet Supplementation Level on Nitrogen Apparent Digestibility and Excretion in Dairy Cows

Yang reported that ruminal infusion of animal fat-coated methionine in beef cattle reduced fecal and urinary nitrogen excretion while significantly improving digestible and retained nitrogen levels, thereby enhancing nitrogen utilization efficiency. Dietary protein utilization efficiency depends primarily on the balance of absorbable amino acids reaching the small intestine, with limiting amino acids being the key influencing factor. Methionine, as one of the limiting amino acids for lactating dairy cows, can be effectively balanced through RPMet supplementation. Methionine deficiency limits the utilization of other amino acids, with excess amino acids being converted to urea via the ornithine cycle in the liver and excreted through the kidneys. When dietary protein level is constant, appropriate RPMet supplementation balances the amino acid profile in the small intestine, facilitating amino acid utilization and reducing urinary nitrogen excretion.

Xie et al. investigated the effects of coated methionine on nitrogen metabolism in Inner Mongolia white cashmere goats, finding that supplementation tended to reduce fecal and urinary nitrogen excretion while significantly improving digestible nitrogen, retained nitrogen, and nitrogen apparent digestibility. Our results similarly demonstrate that RPMet supplementation significantly reduced fecal and urinary nitrogen excretion while significantly increasing digestible nitrogen and nitrogen apparent digestibility. Yan reported that RPMet supplementation significantly reduced urinary nitrogen excretion, tended to decrease fecal nitrogen excretion, and extremely significantly increased nitrogen retention in Small-tailed Han sheep.

Under the conditions of this experiment, dietary RPMet supplementation significantly increased ruminal MCP production, reduced nitrogen excretion, and improved dairy cow performance. Based on comprehensive evaluation of all experimental indices, the optimal RPMet supplementation level was 25 g/(d · head).

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Note: Figure translations are in progress. See original paper for figures.